Assaying the Mutagenic Potential of ELF Radiation through Reverse Mutagenesis via the Ames Test

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The Ames test is a widely accepted, accurate method of testing for mutagenic potential. We thus hypothesized that the Ames test may provide an assay for assessing the mutagenic effects of extra low frequency (ELF) radiation (a subset of EMFs) on certain strains of *Salmonella typhimurium*. The strain of *S. typhimurium* used in the Ames test has a mutation on one gene of the histidine operon which prevents it from growing and replicating without the presence of histidine in the media. When the bacterium is exposed to a mutagen, the defective his gene mutates back to its wildtype state and the bacterium can grow without supplemental histidine. According to Ames tests performed at 1077 volt/meter/AC/E power, 4.6 Gauss AC/B strength, and 3.63 and 5.2 kilo Gauss DC/B power, EMFs appear to have no mutagenic effects on these prokaryotic cells. While it is impossible to test every strength level for all fields and permutations thereof, our tests show that these field strengths failed to demonstrate a mutagenic effect via the Ames test.

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ASSAYING THE MUTAGENIC POTENTIAL OF ELF RADIATION THROUGH REVERSE MUTAGENESIS VIA THE AMES TEST

BY

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ABSTRACT

Electromagnetic Fields (EMFs) exist in many different forms and in a continuum of strengths. They are a common, but unnoticed, part of everyday life. EMFs are produced by the existence of electricity in things ranging from high-tension power lines to the common electrical socket in households. The mutagenic effects, if any, of EMFs has in recent years been widely disputed. While evidence has been presented suggesting mutational effects on cells through exposure EMFs, evidence has also been presented supporting the opposite--no effects whatsoever. Given the paucity of credible causal evidence defining the relationship between EMF and pathogenesis, any relevant data is of significance.

The Ames Test is a widely accepted, accurate method of testing for mutagenic potential. We thus hypothesized that the Ames test may provide an assay for quantifying the mutagenic effects of extra low frequency (ELF) radiation (a subset of EMFs) on certain strains of *Salmonella typhimurium*. The strain of *S. typhimurium* used in the Ames test has a mutation on one gene of the histidine operon which prevents it from growing and replicating without the presence of histidine in the media. When the bacterium is exposed to a mutagen, the defective *his* gene mutates back to its wildtype state and the bacterium can grow without supplemental histidine. According to Ames Tests performed at 1077 volt/meter AC/E power, 4.6 Gauss AC/B strength, and 3.63 and 5.2 kiloGauss DC/B power, EMFs appear to have no mutagenic effects on these prokaryotic cells. While it is impossible to test every strength level for all fields and permutations thereof, our tests show that these field strengths failed to demonstrate a mutagenic effect via the Ames Test.

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INTRODUCTION

Hypothesis

Using the Ames Test, we will test the hypothesis that selected ELFs cause mutagenesis in prokaryotic organisms as a possible model of the mechanism for ELF induced carcinogenesis in humans.

Background

Electromagnetic fields are among the most common constituents of the universe. Radio waves, microwaves, and even light are all comprised of electromagnetic fields (Prata, 1993). The electric currents or potentials in household sockets, power lines, aircraft cockpits, or any electric apparatus produce low-energy electric and magnetic fields that travel at the speed of light. These particular varieties of electromagnetic fields are labeled EMFs. Another name for a particular subset of EMFs is extra low frequency (ELF) radiation. 60-Hertz (Hz) is a common strength for ELF radiation because 60- and 50-Hz are among the most widely used power settings throughout the world (Prata, 1993). 60-Hz is considered a very low oscillation rate compared to other electromagnetic fields, and hence is called ELF radiation.

Electromagnetic fields exist in many different forms. ELF radiation, however, are more narrow in character. ELFs are produced by electric potential or current in wires or

other means of transmission. Current, or the flow of electric charges, can occur in one direction [direct current (DC)], or it can alternate the direction of flow [alternating current (AC)]. Since the majority of ELFs humans are exposed to are produced by the use of electricity, AC and DC ELFs are important to understand.

An example of direct current producing an EMF is a battery hooked up to a copper wire. Essentially, the battery is an electron pump. It pushes electrons into one end of the wire and collects them from the other side. As the electrons move through the wire, they lose energy, most commonly in the form of heat, as they overcome the resistance in the wire. It is important to note that while this process is taking place, the wire does not become charged. The same amount of electrons leave as enter, and there are as many positive charges as negative charges at any point in the wire. However, because many of these negative charges are moving, a magnetic field is produced (see Fig. 1). The ability of the battery or power source to push these electrons through is measured in voltage. Voltage is similar to pressure: the higher the voltage, the more electrons moving through and hence the greater the strength of the magnetic field, also known as a "B field."

Most conventional commercial power systems use an alternating current rather than a direct current. In the AC power system, an electric current flows through the wire. At any given point on the wire, the current and voltage drop

to zero after the charge passes through. Next, the charge reaches the end the circuit and the voltage reverses charge and direction and backtracks its original path. Then the cycle repeats itself over and over. One might think that this would cause appliances to operate in surges, but the process happens too fast to affect appliances (Prata, 1993).

Another type of EMF, electric fields (E-fields), are produced when an electrical potential exists between two juxtaposed points having opposed charges (see Fig. 2).

Both AC and DC power can produce both E- and B-fields. In the following experiments, only the magnetic field, or B-field, was produced from the DC source while both E (electric) and B-fields were generated with AC transformers. The strength of a B-field is measured in units of Gauss or Tesla. The strength of the E-field is directly proportional to both the distance between the charged plates and the power capable of flowing through the wires (voltage).

Concern over EMFs was minimal until two decades ago when scientists began to hypothesize that EMFs were potential health risks (Prata, 1993). Until that point, most scientists believed that EMFs posed very little to no health threat whatsoever. Current research projects attempting to link EMFs to cancer are concentrated mainly in the three areas of epidemiological, *in vivo*, and *in vitro* studies (Prata, 1993). Some of the most conclusive results thus far link childhood leukemia to prolonged exposure to ELFs (See Carpenter, 1994, for a review). Since this new

concern has arisen, the time and money spent on exploring the effects of EMFs has grown exponentially--unfortunately with no concrete answers as of yet. Of particular interest is the mutagenic potential of EMFs on cells, since it is commonly understood that the underlying cause of cancer is a somatic cell mutation that somehow affects the cells ability to regulate its growth. One of the most reliable methods of testing for mutagenesis is the Ames test (Chan, 1993).

In the early 1970's, Dr. Bruce Ames developed a test for mutagenic effects of substances in an attempt to predict potential carcinogens (Russell, 1996). Using strains of *Salmonella typhimurium that* are auxotrophic for the amino acid histidine (*his-*), he tested chemicals which were thought to be carcinogenic. Cells were mixed with a potential mutagen and plated on minimal media (without histidine). Because *his- S. typhimurium* cannot grow in the absence of histidine, only those cells that had undergone a reverse mutation of some sort in the *his* operon would grow on the minimal media. Showing a 90% accuracy with known mutagens, the Ames test was quickly accepted as being the cheapest, most reliable test for mutagens. Though the Ames test can reveal whether something is a mutagen, it does not necessarily identify a carcinogen. This is because carcinogenesis does not always result from a mutation. Thus, the Ames test merely checks for carcinogenic potential (Russell, 1996).

There are many mutant strains of *Salmonella typhimurium* used in the Ames test, each of which are auxotrophic because of a different type of mutation on the *his* operon. All are either frame-shift or base-substitution mutants, and each is designed to test for a different type of reversion or back mutation (Russell, 1996). These mutants are stable and do not commonly revert. The tester-strains also lack a DNA excision-repair mechanism, which would normally correct DNA damaged by a mutagen, thus allowing their use in quantitating mutagenic events. In addition, these mutants have a defective lipopolysaccharide layer on their cell surface that enables mutagens to penetrate into the cell more easily. Certain strains used in this test also carry R-plasmids that render them more susceptible to some weak mutagens.

With that in mind, it is clear that a potential method of testing for the mutagenicity of electromagnetic fields would be the Ames test.

MATERIALS AND METHODS

Media and Solutions

Several different media were used to perform the Ames test. Nutrient broth is prepared, autoclaved at 121° C for 20 minutes, and dispensed into 16x125 mm tubes in 4-5 ml aliquots. The tubes are capped and refrigerated to be used for *S. typhimurium* culturing. Ames test minimal media agar is used as a histidine-deficient media upon which to plate

the bacteria in order to check for reverse mutations. The following are dissolved in 1 liter distilled H_2O, autoclaved at 121° C for 20 minutes, and 20 ml plated on 100mm plates to be refrigerated until needed in the experiment: 15 g agar, 5.2 g $Na(NH_4)HPO_4 \bullet 4H_2O$, 20 g glucose, 15 g K_2HPO_4, 3 g citric acid, 0.3 g $MgSO_4 \bullet 7H_2O$ (Chan, 1993). Finally, 5 g NaCl and 6.5 g agar are dissolved in 1 L distilled H_2O, dispensed into 13x100 mm test tubes in 3-4 ml aliquots, autoclaved for 20 minutes at 121° C, and refrigerated until used as Ames test top agar (Chan, 1993). The top agar is used as a medium to mix the *S. typhimurium*, the biotin/histidine solution, and the mutagen together before placing on the minimal media plate. While experimental procedures call for only 2 ml to coat the minimal media plates, it is important to remember that some liquid is lost in the autoclaving and cooling processes.

The only solution needed for the Ames test is a biotin-histidine solution made by mixing 10.5 mg l-histidine HCl, 12.2 mg biotin, and 100 ml sterile, distilled H_2O (Chan, 1993). Because the HCl lowers the pH of the solution, NaOH must be added until the pH of the solution is 7-8 (tested with pH paper). Once all of the solids have dissolved, the solution is filter sterilized and stored in a sterile container. This solution containing a trace amount of histidine is required to enable all the cells to undergo a few divisions. This is necessary for mutagenesis because

some mutagens act only on replicating DNA. The trace amount of histidine is not, however, enough to allow any colonies to grow.

Bacteria Strains

The only strain of *Salmonella typhimurium* used in this experiment was ATCC #49416, *Salmonella choleraesuis* subspecies *choleraesuis* serotype *typhimurium*. This strain of the bacteria contains base substitution mutation (ATCC Catalog, 1995). The bacterial stock was grown and cultured on either nutrient agar plates or chocolate agar plates. The new cultures were incubated for 48 hours in a 37° C incubator and then placed in a refrigerator for storage until needed to inoculate nutrient broth tubes.

Experimental Procedures

Tubes containing 10-15 ml nutrient broth were inoculated aseptically using one loop of *S. typhimurium* from the stock culture plates. These tubes were then incubated at 37° C, 24 h prior to the beginning of the experiment.

To begin the actual experiment, several preparatory steps were taken. First, the biotin-histidine solution was prepared using the procedure described above. Once that was completed, the top agar tubes had to be heated from their solid state to a liquid state. This was done using a conventional microwave on high power. Because overheating causes the tubes to explode, tubes were heated in short bursts (usually 3-5 seconds). Once the agar is completely

liquefied, the tubes were placed into a water bath set at 45° C. This temperature is warm enough to keep the agar in molten form, but cool enough so as not to kill the bacteria or denature the biotin or histidine.

Once the agar was liquefied and the biotin-histidine solution prepared, the mutagen to be used as a positive control was prepared. In this experiment, the positive control mutagen consisted of a 50/50 mix of methanesulfonic acid ethyl ester and ethanol. Ethanol was used to increase methanesulfonic acid ethyl ester's ability to dissolve in the top agar mixture. Without it, the mutagen would clump around the edges of the petri dishes, thus making the revertant colonies difficult to count. Because the amount of mutagen used in the experiment was very small, only 1 ml methanesulfonic acid ethyl ester and 1 ml ethanol was needed for the mixture. To ensure the sterility of the mutagen solution, Ames minimal media plates were prepared with only the top agar, biotin-histidine solution, and mutagen added. These plates showed no growth, which verified that the mutagen was not contaminated.

To 3.5 ml the top agar, 0.35 ml of the biotin-histidine solution and 0.1 ml 24 hr *S. typhimurium* culture in nutrient broth were added. To the positive control plates, 0.1 ml methanesulfonic acid ethyl ester/ethanol solution was added in addition to the above components. After all the constituents were combined and mixed in the top agar tubes,

they were poured onto one of the Ames test minimal media plates. The experimental plates were then exposed to the ELF within a Faraday Cage. The controls were placed in a Faraday Cage and isolated from the experimental and ambient ELF fields.

In all, four different sets of plates were prepared. The first set consisted of eight plates and was placed into an apparatus capable of generating either AC/B and AC/E fields, depending on which field was being tested. The second set consisted of two plates and was placed into a DC/B field generator. The third and fourth sets were very similar in that they both consisted of three plates each and were used as positive and negative controls. The difference between the positive and negative controls was that the three positive controls had the mutagen added and the three negative controls were otherwise identical to the positive plates except they had no known mutagen added. Neither was exposed to any fields because they were placed in a Faraday Cage. In the second round of experiments, the amount of mutagen and the volume of bacteria were both cut in half for the positive control plates (both from 0.1 ml to 0.05 ml). This was done in order to reduce the number of mutant colonies produced, which was relatively high in the first round.

The eight plates were placed in the AC/E / AC/B field generator. Depending on the experiment, plates were either exposed to an AC/E field of approximately 1100 volts/meter

or an AC/B field of approximately 4.6 Gauss. After the plates were placed into the apparatus, the time and temperatures were recorded. Simultaneously, the six controls were placed in a Faraday Cage to protect them from any fields generated by either the transformers or other sources in the room. The placement time and temperature was recorded for these also.

Finally, the two DC/B field plates were placed into the DC/B field generator. The first setting on the DC/B Field generator was approximately 3.3 kiloGauss (kG), and the second was approx. 5.2 kG. Time and temperature were again recorded and the experiment was allowed to run for 24 h which is sufficient time for reverse mutation to occur based on the positive controls.

After exposure to the experimental conditions for 24 h, the plates were removed from field exposure, time and temperature were again recorded, and the plates were placed into a 37° C incubator for 72 h to facilitate colony growth. After 72 hours in the incubator, the plates were removed and examined for the appearance of colonies, which would indicate a mutational event.

For AC/B field exposure, the exact procedures as above were followed with the only difference being that a magnetic field (B) was used in the AC field generator rather than an electric (E) field.

RESULTS/DISCUSSION

Experimental data are listed in Tables 1 and 2 and displayed on Graphs 1 and 2. No bacteria that underwent field exposure showed any signs of increased colony production when compared to the negative controls.

The positive control plates consistently had between 200 and 650 mutant colonies growing on them as a result of reverse-mutation caused by the known mutagen, methanesulfonic acid ethyl ester.

Negative control plates consistently averaged fewer than 3 colonies. These colonies resulted from a spontaneous reverse-mutation process that cells undergo during reproduction and are called "spontaneous revertants." While many mutations are induced by outside factors, cells also have a tendency to undergo spontaneous mutations as a result of random replication errors. In this case, a spontaneous mutation on the histidine operon of an *S. typhimurium* cell would allow the cells to grow without the presence of histidine on the minimal media plates.

We conclude the electromagnetic fields of the types and strengths tested do not have a quantifiable mutagenic effect on cells of one strain of *S. typhimurium* in the Ames test. In every case, the number of mutated colonies growing on plates that were exposed to EMFs was extremely close to the number of spontaneous revertants on the negative control plates. The difference between the positive controls and the experimentals is significant to the $P = <0.05$ level

with no significant difference between the negative controls and the experimentals.

It is important to note, however, that although we did not observe increased mutagenesis, this does not mean that ELF is not mutagenic. Different field strengths may have different effects on the cells and it is therefore important to continue the research in this area. Also, there is the possibility of a resonance effect at certain field strengths and types that could cause mutation. It is also important to note that *S. typhimurium* ATCC #49416 has only one specific type of mutation. It may be that ELF radiation can cause mutations of a different types of base substitutions or frameshifts. Some types could be assayed by different strains of *S. typhimurium*, but others such as chromosomal rearrangement mutations called inversions, deletions, etc. can not be assayed by the Ames test (Tamarin, 1991). Recommendations for future research include the study of both combined field exposure and the resonance effects done on different strains of *S. typhimurium* containing all possible mutational categories. It would also be important to expand this research into eukaryotic systems, the most pertinent being human cell lines.

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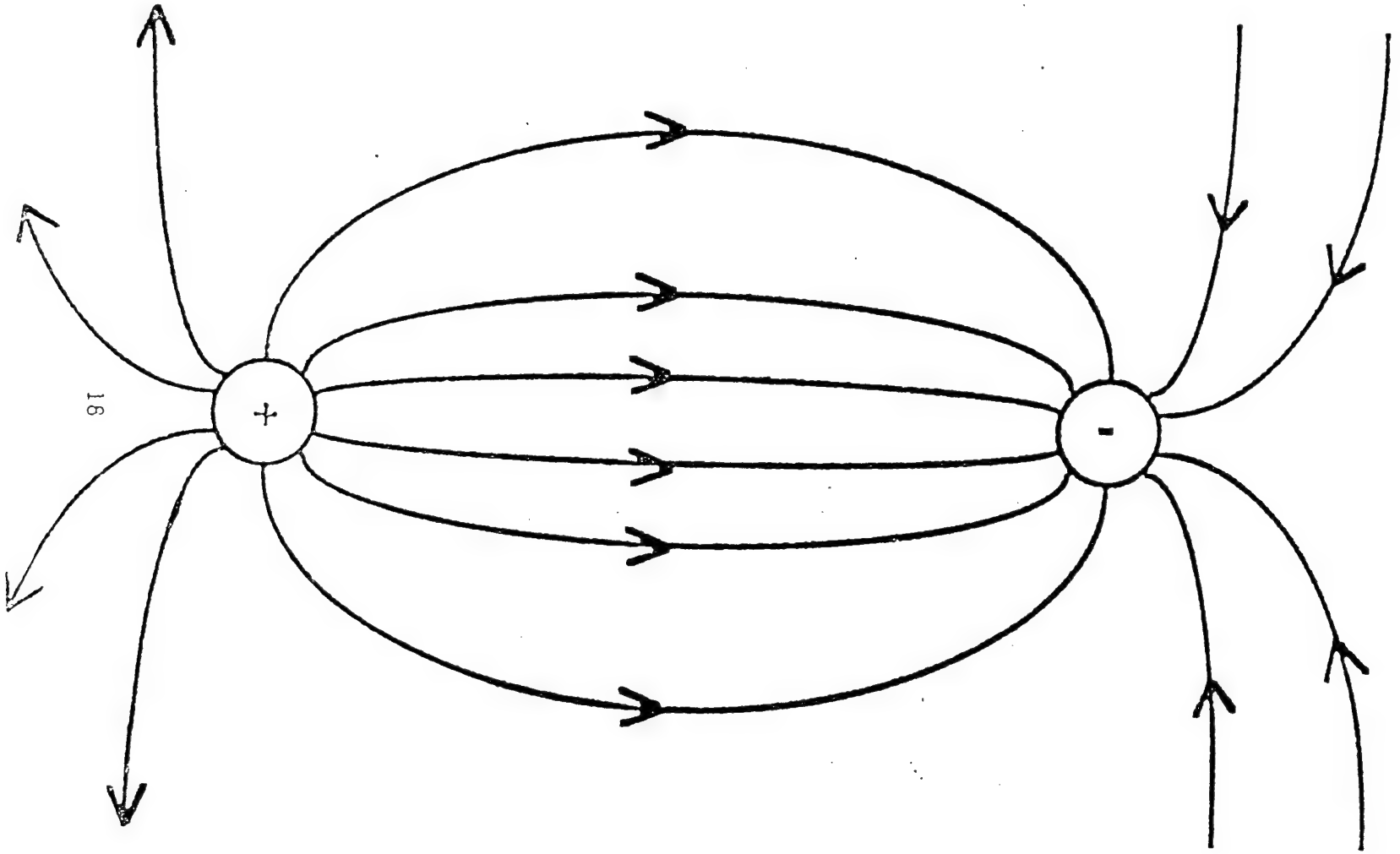
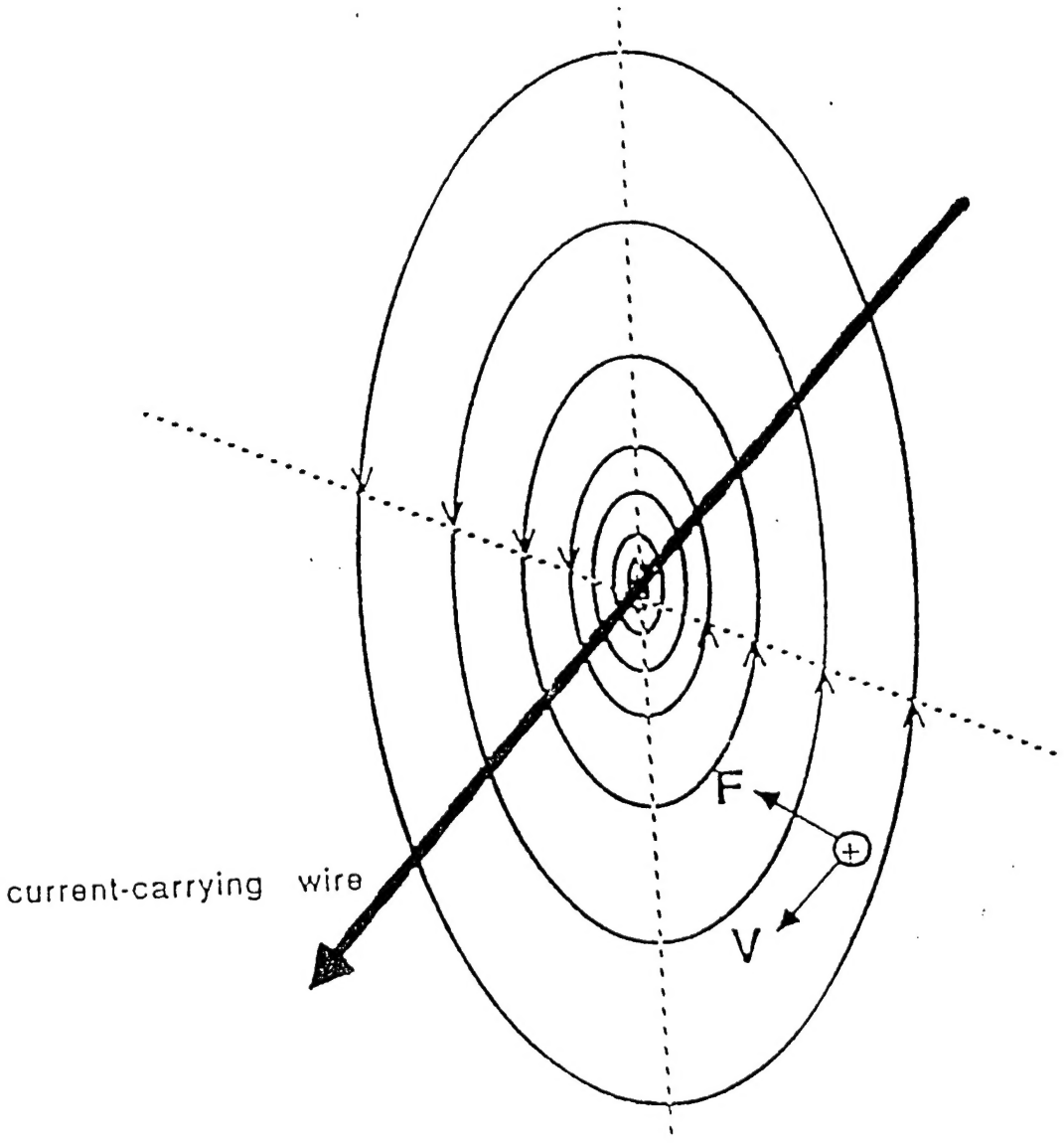


FIGURE 1

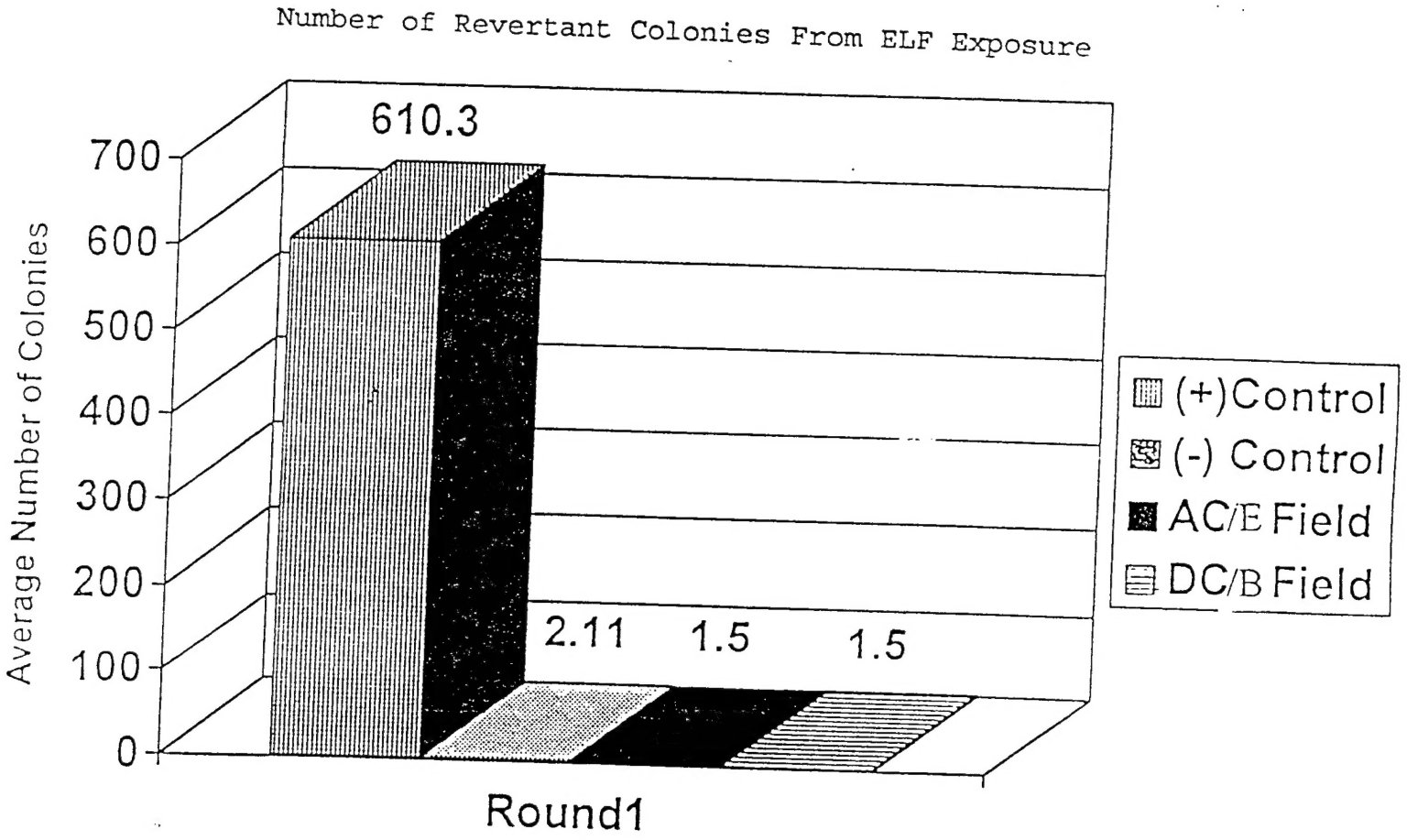
The electric field of two equal but opposite charges. A small positively charged particle placed somewhere in this field will experience a force in the direction of the local field line (note arrows). The strength of the force is proportional to the spacing between the field lines (field lines closer together means a higher field and thus a larger force on small charged particle). (U. S. Congress 1989)

FIGURE 2



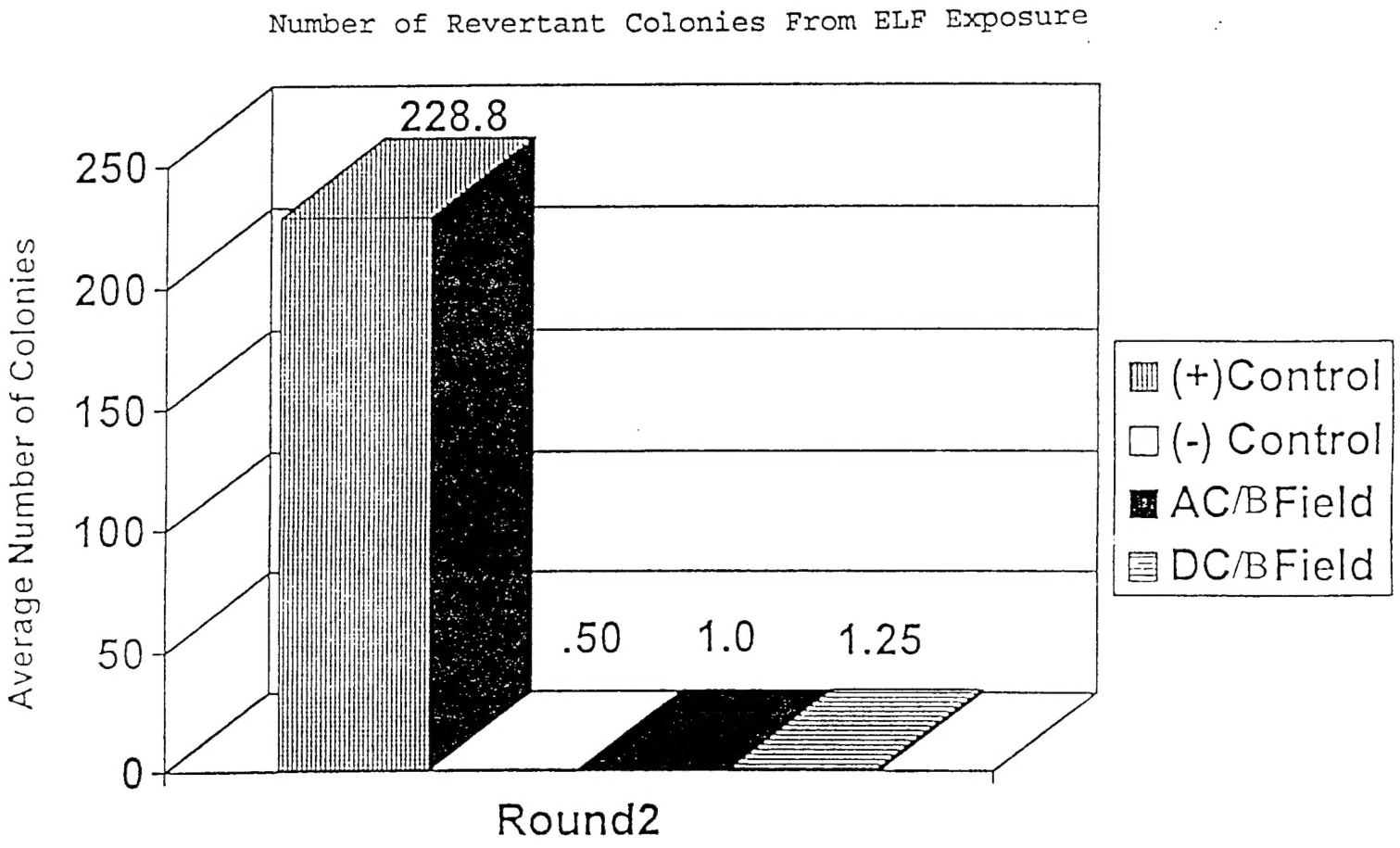
The magnetic field of a long straight wire produces a force, F, on a positively charged particle that is moving nearby. The strength of the field is proportional to the spacing between the lines (closer spacing means stronger field). The direction of the magnetic force on a charged particle moving in the field is perpendicular to both the field lines and the particle's direction of motion, V. (U. S. Congress, 1989).

Graph 1



Graph 1: Bar graph showing the results of the first round of experiments. The large bar on the left depicts the positive control to which the known mutagen was added. Results strongly indicate no mutational effects by exposure to fields.

Graph 2



Graph 2: Bar graph representation of the second round experiment results. Bar on far left depicts positive control that had known mutagen added. Once again, these results suggest no mutational effects after exposure to ELF radiation.

Table 1 A

	Temp (C)	Time (hrs	Strength	Colonies	Average
(+) Contr	24	24	N/A	552, 594, 626	590.667
(-) Contr	24	24	N/A	2, 3, 2	2.33
AC/E	25	24	1100 volts/	1, 2, 2, 1, 0, 2, 3, 1	1.5
DC/B	24	24	3.34 kGauss	0, 2	1

Table 1B

	Temp (C)	Time (hrs	Strength	Colonies	Average
(+) Contr	23	24	N/A	604, 592, 633	609.667
(-) Contr	23	24	N/A	1, 2, 2	1.667
AC/E	25	24	1085 volts/	2, 2, 0, 1, 3, 2, 2, 1	1.625
DC/B	25	24	3.23 kGauss	2, 2	2

Table 1C

	Temp (C)	Time (hrs	Strength	Colonies	Average
(+) Contr	22	24	N/A	619, 648, 625	630.667
(-) Contr	22	24	N/A	3, 3, 1	2.333
AC/E	22	24	1084 volts/	1, 1, 1, 1, 2, 2, 1, 2	1.375
DC/B	24	24	3.41 kGauss	1, 2	1.5

Tables 1A-1C and 2A-2B: Tabular depiction of data collected in the experiments. Each number under "Colonies" represents the growth on one plate. Numerical source for Graph 1 and Graph 2.

Table 2A

	Temp (C)	Time (hrs	Strength	Colonies	Average
(+) Contr	24	24	N/A	221, 247, 223	230.333
(-) Contr	24	24	N/A	1, 0, 1	0.667
AC/B	36	24	4.6 Gauss	2, 1, 1, 1, 1, 0, 0, 1,	0.875
DC/B	23	24	5.23 Gauss	1, 1	0.667

Table 2B

	Temp (C)	Time (hrs	Strength	Colonies	Average
(+) Contr	24	24	N/A	230, 247, 223	233.333
(-) Contr	24	24	N/A	0, 0, 1	0.333
AC/B	36	24	4.5 Gauss	0, 0, 2, 2, 1, 1, 1, 2	0.875
DC/B	23	24	5.78 kGauss	2, 1	1